PULMONARY OXYGEN TOXICITY--AUGMENTATION OF ENDOGENOUS DEFENSE SYSTEMS

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Many pathological conditions have been described where tissue damage due to overproduction of partially-reduced species of oxygen overwhelms tissue defense systems. Oxygen radicals have been implicated in tissue injury due to activated leukocytes, treatment with quinoid antibiotics and anthracyclic chemotheraupeutic agents, paraquat poisoning, ionizing radiation, ischemia and hyperoxia.

We have previously shown that hyperoxia causes enhanced rates of production of the toxic oxygen species O_2^- and H_2O_2 in lung cells. During pulmonary hyperoxia, these oxygen species can be produced by subcellular organelles, which include mitochondria, endoplasmic reticulum and the nuclear membrane. Polymorphonuclear leukocyte infiltration due to oxidant lung damage can also result in extracellular production of leukocyte-derived oxygen radicals. Since enzymes such as superoxide dismutase and catalase protect cells from damage by these oxygen species, a method has been developed to augment these endogenous lung defenses. Superoxide dismutase and catalase have been entrapped in liposomes consisting of phosphatidylcholine, cholesterol and stearylamine or dicetyl phosphate, if positively or negatively charged liposomes are desired. Liposomes can mediate intracellular delivery of normally membrane-impermeable substances via processes of fusion and endocytosis.

The biological effect of antioxidant enzyme-containing liposomes have been tested in three systems: cultured aortic endothelial cells, isolated perfused lungs and rats. Cultured endothelial cells serve as a model for the oxygen-sensitive capillary endothelial cell of lungs. Treatment of confluent monolayers of endothelial cells with liposomes results in up to a 100-fold increase in cell-associated antioxidant enzyme activity. The liposome-enhanced superoxide dismutase and catalase activities are stable up to two days after treatment, when cells become no longer appropriate for study. Measurement of (^{51}Cr) and lactate dehydrogenase release by cells into the culture medium showed that cell integrity is maintained during and after liposome-mediated enzyme augmentation. Cells grown at various oxygen tensions released (^{51}Cr) and LDH into culture medium in increasing amounts as a function of oxygen concentration, indicating oxidant cell damage. Cells treated with superoxide dismutase or catalase-containing liposomes released less (^{51}Cr) or LDH than controls at all oxygen tensions. Controls included untreated cells, cells treated with free enzymes, and liposomes containing inactivated enzyme. This shows that liposome-mediated antioxidant enzyme augmentation protects cultured cells from oxygen injury.

Intravenous injection of liposomes containing catalase and SOD increased lung enzyme specific activities. Lungs removed from liposome-treated rats and perfused with xanthine plus xanthine oxidase or phorbol myristate acetate-stimulated leukocytes had a significantly decreased edematous response to perfusate-generated free radicals. Liposome treatment also increased the survival time of rats exposed to 100% oxygen. Intravenous injection of control liposomes or free enzymes had no effect on survival time of rats in 100% oxygen. These results show that pulmonary oxygen toxicity is directly related with an enhanced rate of production of reduced oxygen species and that liposomes containing antioxidant enzymes can be used for modification of pathologic states where oxygen radical formation is problematic.